# THE INFLUENCE OF GLANDULAR EXTRACTS UPON THE CONTRACTILE VACUOLES OF PARAMECIUM CAUDATUM.

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Contractile vacuoles have long been puzzling morphological elements in the Infusoria. In general they may be said to be cavities in the protoplasm destined to expel by contraction the liquid which they accumulate. They are present in all ciliated infusorians except a few salt water and parasitic forms, but since this paper deals primarily with the physiology of the vacuoles in *Paramecium caudatum* a description of their structure will be limited to this species.

Normally *Paramecium caudatum* possesses two contractile vacuoles situated in the ectoplasm, one at the posterior end and the other about mid-way between the anterior and posterior ends. Cases have been noted by Hance (11) where a race seemed to show a tendency toward multiple vacuoles, and by Shumway (26) where the individual appeared to be stimulated to the production of extra vacuoles by some abnormal physiological influence. Invariably the vacuoles are arranged in a straight line. Each vacuole is composed of a central reservoir with peripheral canals radiating from it, the number of canals varying with the individual vacuole. The mechanism of contraction is as follows. The canals swell slowly, then discharge their fluid contents into the reservoir, which in turn contracts, expelling its contents to the outside through an excretory pore. In systole the reservoir disappears completely from view, leaving clearly visible the rosette of canals slowly filling again. A new cavity must be formed by contraction of the canals in the protoplasm which has replaced the old reservoir. The outline of this new reservoir is irregular at first, but gradually assumes the shape of the circular vacuole. The canals are simple afferent tubules which pulsate twice to every contraction of the vacuole and

remain constant in form and structure. While the reservoir appears always in the same place, its walls are formed of new substance at each pulsation. There is much controversy as to the exact nature of the enveloping material, Kent (16) cited Carter, Ehrenberg, Siebold, Claparede and Lachmann as believing in the presence of a morphologically distinct investing membrane, while Maupas (20), Ehrmann (8), Degen (6), and Minchin (21) upheld the conflicting view that the vacuole is simply a drop of watery fluid lodged in, and bounded by a more viscid protoplasm. Maupas found the wall comparable to that of a soap-bubble, since according to him, the viscid protoplasm is distended by increased pressure until it breaks at the weakest point, the excretory pore. The rhythmic contractions he believed due merely to the innate irritability of the protoplasm in response to a specific stimulation. According to Bütschli's alveolar theory of protoplasm (3) the behavior of the vacuoles is explicable entirely by the physical laws of fluid masses. Andrews (1) developed the application of this theory, describing the rhythmic variation of viscosity in the pellicular membrane of the contractile vacuole:

"At times in the rhythm the vacuole actually disappears, and during collapse the pellicular substance becomes so completely relaxed, so fluid, that it mingles with the interalveolar substance of the surrounding protoplasm. As the membrane reforms after such collapse it thickens and is gradually augmented by interalveolar stuff. As a contractile pellicle it is subject at all times to the same flow of its substance as is the surrounding network, and is at times so modified in structure and reaction as to form an area of organized physiological reaction."

Claparede and Lachmann believed that the vacuole has no intercommunication with the surrounding medium, while Carter and Lankester (18) held that the vacuole discharges externally at the time of collapse. Wrzesniowski (30) Maupas (20), Butschli (3) and Hance (11) definitely described the pore-like character of the communication. Jennings (14) proved this inter-communication conclusively by demonstrating the discharge of the vacuole into a surrounding medium of dilute india ink.

The theory of Haeckel (10) that the pulsating vacuoles are derived from the simple food vacuoles was questioned by Maupas (20), who declared that the two vacuoles have but one characteristic in common,—the absence of a true limiting membrane. In all other characteristics they differ completely. The fluid content of the contractile vacuole contains the soluble waste products of metabolism, while that of the food vacuole is a plasma or cellulose sugar in which digestion can take place. According to Maupas (20) the contractile vacuole is a special physiological adaptation of the protoplasm and does not depend phylogenetically upon any other structure.

Opinions are even more contradictory in regard to the special function of the vacuole. Hartog (12) and later Stempell (28) emphasized the importance of the vacuole as an adjustor of osmotic equilibrium. It is a physical necessity to the naked cell living in water since it prevents overdilution and ultimate destruction of the protoplasm. Others ascribed to the vacuole the additional functions of circulation, respiration, and excretion. No one can doubt its assistance in general circulation. Kent (16) mentioned that Lieberkuhn, Claperede and Lachmann even considered it a rudimentary heart. In respiration it serves according to Haeckel (10), Maupas (20), Butschli (3), Ehrmann (8) and Stempell (28) merely as an agent to remove the end products of oxidation. The fluid which accumulates in the vacuole has previously circulated through the organism, and must therefore have lost the feeble quantity of oxygen which it held in solution on entering the protoplasm through the gullet or the periphery. The vacuole may be said to receive all the soluble end products of general metabolism. Schmidt (16) found the vacuole comparable in function to the renal organs of higher animals, especially the excretory water canals of *Turbellaria*.

Rossbach (24) first showed conclusively that the rhythm and size of the vacuole vary with the physical and chemical properties of the surrounding medium. Since then a considerable amount of work has been done on the physiology of these primitive organs. The pulse frequency, or the number of seconds elapsing between two successive pulsations presents under normal conditions a constant average among individuals of the same race.

Ehrmann (8) set this normal average as twelve for *Paramecium*. Hance (11) found that with multiple vacuoles there is an increase in pulse frequency toward the posterior end. The observations reported in this paper will show however that this may occur also in normal individuals. Moreover, the reverse is frequently true, that the anterior vacuole or vacuoles may pulsate more rapidly than the posterior. Rossbach (24) experimenting with temperature changes, gases, chemical solutions and electricity found that rate of contraction increased with rise in temperature and with most chemical reagents. Dilation with retardation was produced by neutral substances and alkaloids, and no effect whatever was noticeable with currents of oxygen or of electricity. Korentschewsky (17) confirmed this retarding effect of alkaloids.

Degen (6) published an exhaustive treatise on the physiology of the vacuole. Continuing the temperature experiments of Rossbach, he established a maximum and minimum for the organism. Around zero degrees he found a great variation in pulse frequency, but a generally slow contraction with dilation of the vacuole. At about three, Centigrade, dilation ceased and the pulse frequency became more and more rapid until the animal plasmolysed at about thirty-four degrees. Doflein (7) gave the optimum temperature as thirty to thirty-five degrees for *Paramecium*. In contrast to Rossbach's observations (24) Degen found that a current of oxygen produced a pulse quickening to compensate for the increased output of carbon dioxide produced by the accelerated metabolism. Hydrogen, however, retarded the pulse, dilated the vacuole and caused dissolution in four or five hours. When subjected to carbonic acid gas the vacuole dilated with an increase in pulse frequency and dissolution occurred in two hours. Minchin (21) observed that carbon dioxide diminished the frequency. This seeming contradiction may be due to the specific resistance of the organism in media saturated with the gas. Jacobs (13) believes that the organisms in such environment develop a remarkable resistance to other chemical factors. That pulse frequency often may be attributable to the concentration of the surrounding medium was shown by Degen in the effects of chemical solutions of varying strength.

An important factor is the specific permeability of the osmotic membrane. Degen found that alcohol, ether, acetone and chloroform had a rapid lethal effect but little direct influence on the vacuole. He concluded from his various experiments that the vacuolar membrane has a remarkable physiological, but no morphological differentiation from the surrounding protoplasm. The frequency of the pulse he believed conditioned by the water intake and its osmotic value relative to the environmental fluid.

It would seem that any agent as powerful as the glandular extracts should exert a strong influence upon the rhythmic contractions and perhaps by means of a specific influence give some important information as to the function of this primitive organ. Some work has been done already on the effect of the extracts upon the general metabolism of protozoa. Nowikoff (22) found that thyroid extract increased the division rate in *Paramecium*. Extract of hypophysis had no such effect. Calkins (4) obtained no reactions with pancreatic extract. Shumway (26) confirmed Nowikoff's observations, finding that thyroid extract was the only glandular product producing any significant increase in division rate. He also noted a tendency toward the formation of extra vacuoles and an increase in pulse frequency. Chambers (5) found that a solution of suprarenal extract with a basic fluid of either hay infusion or malted milk produced an increase in division rate, while a solution of pituitary, Armour & Co. tablets, produced no influence with malted milk and only a very slight increase in division rate with hay infusion. A mixture of pituitary and suprarenal solutions in the basic food substance appeared to reduce the division average.

In October, 1918, the following experiments were started to determine the specific influence of adrenalin, pituitary substance and pineal gland extract upon the contractile vacuoles of *Paramecium caudatum*. Individuals for the experiment were taken from wild cultures just introduced into the laboratory. First a number of observations were made upon pure line cultures to determine whether or not there might be, (1) any significant variation in the pulse frequency of individuals in different pure lines kept in the same physiological environment, (2) any significant similarity in the pulse frequency of individuals in the

same pure line kept in the same physiological environment, and (3) any significant diversity in the pulse rate of individuals from a wild culture kept under approximately the same conditions in the laboratory. Two pure lines—A and B—were started on a depression slide, each being given 2 drops of distilled water and one drop of 1 per cent. malted milk solution daily. On another depression slide were placed drops containing individuals taken from a wild culture. These were given the same diet. Both slides were kept in a moist chamber at room temperature. On the third and fourth days averages were made of the pulse rate of four individuals from each group. It was found by repeated experimentation that when the animals were fed at ten o'clock in the morning they usually quieted down at about four o'clock in the afternoon. Various futile attempts were made to quiet them artificially without disturbing the pulse rate, but any methods employed which increased the density or the pressure of the surrounding medium definitely decreased the pulse frequency. The individuals were kept on depression slides and observed under both high and low power. By using a stop-watch an accurate record of the pulsations could be kept. In all cases the pulse rate of both vacuoles was taken, going alternately from one to the other, and recording ten pulsations of each. It would be unwise to observe ten contractions of one vacuole before noting the other because of the rapid effect of any change in physiological conditions. Tables I. and II. give the results of the first series of experiments.

From these records it is seen that the variability in pulse frequency in different pure lines in not significantly greater than the variability among individuals in a single pure line. Taking the anterior vacuole, the average for pure line A is 3.9 seconds more rapid than for pure line B, while the greatest variation for any group within pure line A is 1.6 and for any group within pure line B is 4.3. The same is true for the posterior vacuole. The average for pure line A is 3.5 seconds more rapid than for pure line B, while the greatest variation for any group within pure line A is 1.8 and for any group within pure line B is 4.1. For the wild culture the average of each vacuole presents approximately the mean average of the corresponding vacuoles of the

two pure lines, and the variations among individuals from the culture are not so great as the variations among ind viduals within either pure line. From this we may conclude that there is (1) no significant variation in the pulse frequency of individuals in different pure lines kept in the same physiological environment, (2) no significant similarity in the pulse rate of individuals in the same pure line kept in the same physiological environment, (3) no significant diversity in the pulse rate of individuals from a wild culture kept under approximately the same conditions in

TABLE I.

PULSE FREQUENCY UNDER NORMAL CONDITIONS.

*(Given in Seconds.)*

*Pure Line A.*

| 1. | | 2. | | 3. | | 4. | |
|---|---|---|---|---|---|---|---|
| Ant. | Post. | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 13.0 | 11.0 | 13.0 | 10.6 | 9.0 | 8.0 | 12.0 | 11.2 |
| 13.0 | 14.0 | 13.0 | 10.6 | 9.8 | 8.2 | 11.4 | 11.2 |
| 13.0 | 12.5 | 11.5 | 13.0 | 9.8 | 9.4 | 12.4 | 10.6 |
| 12.0 | 11.0 | 11.2 | 10.6 | 10.0 | 9.2 | 12.0 | 10.2 |
| 13.0 | 13.0 | 11.2 | 11.0 | 10.0 | 9.0 | 11.4 | 11.2 |
| 12.0 | 11.0 | 12.0 | 10.6 | 9.6 | 8.4 | 11.0 | 11.0 |
| 13.0 | 12.0 | 12.0 | 10.4 | 9.8 | 9.2 | 12.6 | 11.0 |
| 11.5 | 12.0 | 11.2 | 12.0 | 9.4 | 8.8 | 11.0 | 10.8 |
| 11.5 | 12.5 | 12.0 | 12.0 | 9.6 | 9.8 | 10.0 | 10.4 |
| 11.0 | 10.5 | 12.0 | 11.0 | 9.8 | 9.0 | 10.0 | 12.0 |
| 123 | 119.5 | 119.1 | 111.8 | 96.8 | 89 | 113.8 | 109.6 |
| 12.3 | 11.9 | 11.9 | 11.1 | 9.6 | 8.9 | 11.3 | 10.9 |

*Pure Line B.*

| 1. | | 2. | | 3. | | 4. | |
|---|---|---|---|---|---|---|---|
| Ant. | Post | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 17.0 | 17.0 | 15.0 | 17.0 | 16.6 | 14.0 | 10.6 | 10.2 |
| 19.4 | 18.6 | 17.0 | 17.6 | 16.4 | 14.0 | 10.6 | 10.4 |
| 17.0 | 16.6 | 17.4 | 15.0 | 16.0 | 14.2 | 10.4 | 9.8 |
| 18.0 | 16.8 | 16.2 | 15.8 | 15.0 | 12.4 | 11.2 | 10.4 |
| 17.0 | 17.2 | 17.6 | 14.0 | 17.0 | 12.4 | 10.6 | 9.8 |
| 17.6 | 16.6 | 16.2 | 14.0 | 16.4 | 15.2 | 11.0 | 9.8 |
| 17.4 | 17.6 | 15.4 | 14.4 | 17.0 | 14.2 | 10.8 | 10.2 |
| 17.0 | 17.0 | 16.0 | 14.0 | 16.0 | 16.6 | 10.8 | 10.8 |
| 17.0 | 15.8 | 14.8 | 15.6 | 15.0 | 15.2 | 11.2 | 10.4 |
| 17.0 | 17.0 | 16.6 | 17.0 | 16.0 | 15.0 | 10.8 | 10.0 |
| 174.4 | 170.2 | 162.2 | 154.4 | 161.4 | 143.2 | 108.0 | 101.8 |
| 17.4 | 17.0 | 16.2 | 15.4 | 16.1 | 14.3 | 10.8 | 10.1 |

*Wild Culture.*

| 1. | | 2. | | 3. | | 4. | |
|---|---|---|---|---|---|---|---|
| Ant. | Post. | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 11.4 | 10.6 | 14.4 | 12.6 | 10.8 | 11.0 | 12.8 | 11.0 |
| 12.0 | 11.2 | 14.4 | 12.2 | 10.4 | 11.0 | 12.6 | 11.0 |
| 13.0 | 12.0 | 15.0 | 12.4 | 11.0 | 11.2 | 13.0 | 11.4 |
| 12.0 | 11.2 | 14.4 | 12.4 | 11.2 | 10.8 | 13.2 | 11.4 |
| 12.0 | 11.6 | 14.6 | 13.0 | 10.8 | 11.2 | 13.2 | 11.4 |
| 13.0 | 11.8 | 15.0 | 12.6 | 11.2 | 11.8 | 13.0 | 11.0 |
| 13.0 | 11.2 | 15.0 | 12.6 | 11.0 | 12.0 | 13.0 | 11.0 |
| 13.4 | 12.0 | 14.4 | 12.2 | 10.0 | 11.2 | 12.6 | 11.2 |
| 12.6 | 12.4 | 14.4 | 13.0 | 11.2 | 10.8 | 13.0 | 11.0 |
| 12.0 | 12.0 | 14.4 | 12.2 | 11.0 | 11.0 | 12.8 | 12.8 |
| 123.4 | 116.0 | 146.0 | 125.2 | 108.6 | 121.0 | 129.2 | 113.2 |
| 12.3 | 11.6 | 14.6 | 12.5 | 10.8 | 11.2 | 12.9 | 11.3 |

## Table II.

Average Rate of Pulsation for Each Group.

| Anterior Vacuole. | | Posterior Vacuole. | |
|---|---|---|---|
| Pure line A | 11.2 | Pure line A | 10.7 |
| Pure line B | 15.1 | Pure line B | 14.2 |
| Wild Culture | 12.6 | Wild Culture | 11.6 |

the laboratory, and (4) there is an average rate for all Paramecia kept under the same physiological conditions. In this case the average for the anterior vacuole is 12.9 and for the posterior vacuole 12.1. These figures are remarkably close to the normal average set by Ehrmann (8) which was 12.

In the experiments with glandular extracts individuals from various wild cultures were used. Since the pulsations do not seem to be subject to specific pure line characteristics it was thought wiser to work with individuals not kept for any length of t'me in the artificial environment of distilled water and malted milk. It has already been shown that Paramecia taken from a wild culture in spr'ng water, and placed on depression slides with 2 drops of distilled water and 1 drop of 1 per cent. malted milk solution daily show after three and four days of this treatment in a moist chamber at room temperature a perfectly normal rate of pulsation. The following glandular preparations of Parke, Davis & Co. were used—adrenalin in crystal form, pituitary substance made from the desiccated anterior lobe of the pituitary body, and pineal gland tablets prepared from the desiccated

gland. Each of these preparations produces a specific effect upon higher organisms, the first contracting the arteries and capillaries, raising the blood pressure, and stimulating the heart, the second acting as a powerful stimulant to growth, and the third slightly increasing the blood pressure. The object of the following experiments is to discover whether these glandular products have an equally specific effect upon the pulse frequency of the contractile vacuole. The preparations were made up with distilled water into solutions of varying strengths, 1–2,000,000, 1–1,000,000 and 1–200,000. Observations were made immediately after adding drops of a solution to the culture medium. The results of the experiments are shown in the tables.

TABLE I.

EFFECT OF ADRENALIN, 1–2,000,000.

| Normal Conditions. | | 1 Drop Adrenalin, 1–2,000,000. | |
|---|---|---|---|
| Ant. | Post. | Ant. | Post. |
| 12.0 | 12.2 | 9.8 | 11.4 |
| 9.8 | 10.2 | 9.0 | 11.4 |
| 10.2 | 10.0 | 10.2 | 10.0 |
| 10.4 | 9.4 | 9.0 | 11.0 |
| 10.2 | 11.0 | 9.2 | 11.0 |
| 10.1 | 11.6 | 10.0 | 12.2 |
| 9.8 | 12.2 | 10.0 | 10.0 |
| 10.2 | 12.2 | 9.4 | 9.2 |
| 10.2 | 11.0 | 9.4 | 11.0 |
| 10.2 | 11.4 | 9.8 | 11.0 |
| 103.1 | 111.2 | 95.8 | 108.2 |
| 10.3 | 11.1 | 9.5 | 10.8 |

The increase in pulse frequency after the addition of one drop of adrenalin, 1–2,000,000 is insignificant. No greater increase occurred at any time within two hours after the treatment with adrenalin.

Fig. 1 represents graphically the effect of adrenalin upon the vacuoles of a *Paramecium* in an abnormal physiological condition, produced by some unknown factor. Treatment with one drop of adrenalin, 1–2,000,000 solution did not prevent the rapidly diminishing frequency. One drop of adrenalin, 1–1,000,000 solution produced an immediate quickening in both vacuoles,

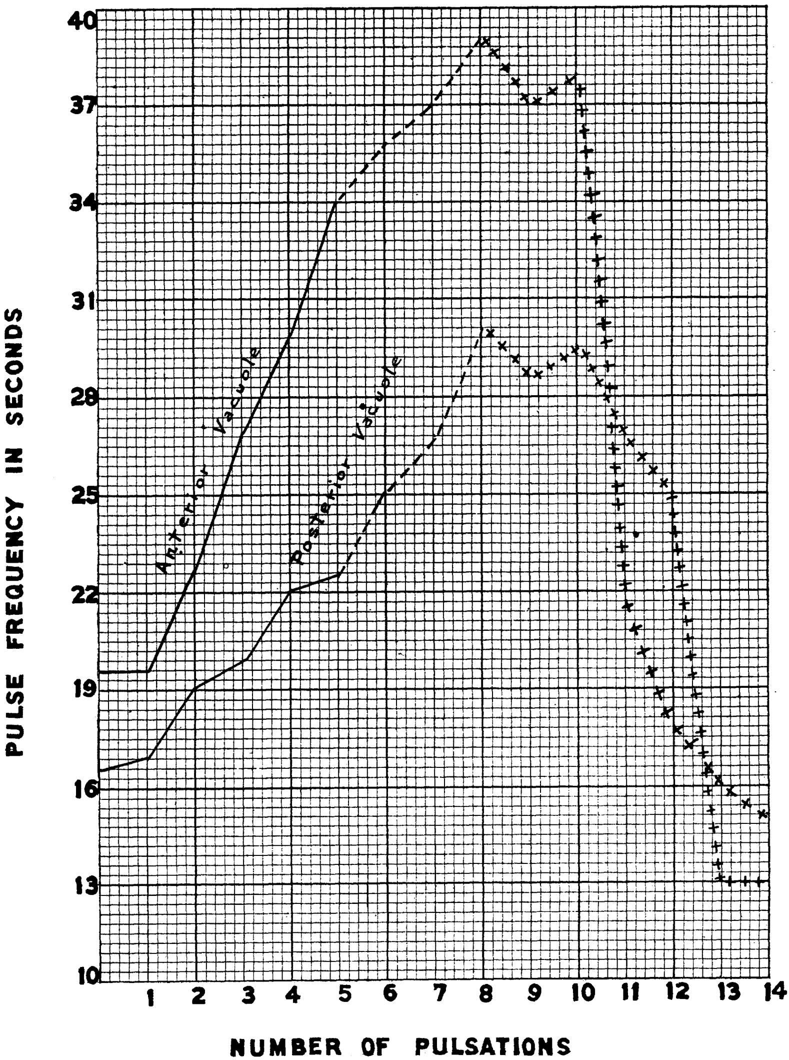


FIG. 1. Increase in pulse frequency following treatment with adrenalin. Ordinates represent pulse frequency in seconds, abscissæ, the number of pulsations recorded. Unbroken lines indicate pulsations previous to treatment with adrenalin. Broken lines indicate pulsations after treatment with 1 drop of adrenalin, 1 : 2,000,000 solution. Lines of crosses indicate pulsations after treatment with 1 drop of adrenalin, 1 : 1,000,000 solution.

continuing until a practically normal rate of pulsation was restored. However, the effect was not lasting. Observations made fifteen minutes later showed a rate of 50 for the anterior, and 42 for the posterior vacuole.

Table II. gives a detailed record of the reaction to adrenalin-1–200,000 solution, in two individuals. From these results and

TABLE II.

EFFECT OF ADRENALIN, 1 : 200,000.

| Normal Conditions. | | | | 1 Drop Adrenalin, 1 : 200,000. | | | |
|---|---|---|---|---|---|---|---|
| A. | | B. | | A. | | B. | |
| Ant. | Post. | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 13.0 | 19.0 | 11.0 | 12.2 | 13.2 | 15.0 | 10.2 | 12.0 |
| 12.8 | 18.6 | 10.0 | 12.0 | 11.2 | 15.0 | 8.2 | 11.0 |
| 14.4 | 20.0 | 12.0 | 12.0 | 13.0 | 14.4 | 7.6 | 10.2 |
| 16.0 | 20.2 | 12.2 | 12.0 | 13.2 | 14.4 | 8.0 | 9.2 |
| 15.0 | 20.0 | 11.2 | 11.4 | 13.0 | 14.0 | 8.0 | 9.0 |
| 15.2 | 19.2 | 9.8 | 12.0 | 13.0 | 14.0 | 8.2 | 9.0 |
| 14.6 | 18.8 | 12.4 | 12.4 | 13.0 | 13.2 | 8.6 | 12.8 |
| 15.2 | 20.6 | 11.0 | 10.6 | 13.0 | 14.0 | 9.6 | 13.0 |
| 15.0 | 18.2 | 9.2 | 12.2 | 12.0 | 13.0 | 12.0 | 13.0 |
| 15.0 | 19.6 | 11.0 | 11.2 | 12.2 | 13.4 | 12.0 | 12.0 |
| 146.2 | 194.2 | 109.8 | 118.0 | 125.8 | 140.4 | 92.4 | 111.2 |
| 14.6 | 19.4 | 10.9 | 11.8 | 12.5 | 14.0 | 9.2 | 11.1 |

many other similar ones it seems probable that adrenalin of the above strength has a specific quickening effect upon the contractile vacuole. This effect is usually of very short duration, especially if it produces an abnormally rapid rate of pulsation.

Treatment with adrenalin, 1–200,000 solution, produces also a marked dilation of the vacuoles which persists for some time after the rate of contraction has returned to normal. From several records there are indications that the duration of the pulse acceleration is prolonged by increase in the strength of the solution up to a certain point which has not been exactly determined. In three individuals one drop of adrenalin, 1–10,000 solution proved fatal. Not enough records with intermediate strengths have been taken to warrant any definite assumption of a specific optimum strength.

It is evident from the records in Table III. that the effects

## TABLE III.

EFFECTS OF ADRENALIN ON AN INDIVIDUAL WITH THREE VACUOLES.

| Normal Conditions. | | | 1 Drop Adrenalin, 1 : 200,000. | | |
|---|---|---|---|---|---|
| Pre-Ant. | Ant. | Post. | Pre-Ant. | Ant. | Post. |
| 19.6 | 17.0 | 17.0 | 18.0 | 17.0 | 17.6 |
| 20.0 | 16.2 | 17.0 | 17.0 | 15.0 | 13.0 |
| 20.0 | 17.2 | 17.0 | 19.0 | 16.0 | 14.0 |
| 18.0 | 17.0 | 18.0 | 18.4 | 16.4 | 14.2 |
| 21.0 | 16.8 | 17.8 | 19.0 | 15.2 | 15.0 |
| 18.8 | 17.0 | 18.2 | | 14.2 | 14.0 |
| 19.2 | 16.4 | 18.2 | | 14.0 | |
| 19.6 | 16.4 | 17.0 | | 13.8 | |
| 20.0 | 17.0 | 17.0 | | 14.0 | |
| 19.8 | 17.0 | 17.8 | | 13.8 | |
| 196.0 | 16.80 | 175.0 | 91.4 | 149.4 | 87.8 |
| 19.6 | 16.8 | 17.5 | 18.2 | 14.9 | 14.5 |

of adrenalin on an individual with three vacuoles do not differ from the effects on an individual with two vacuoles.

While the records in Table IV. do not represent a complete

## TABLE IV.

EFFECT OF PITUITARY, 1 : 2,000,000.

| Normal Conditions. | | 1 Drop Pituitary, 1 : 2,000,000. | |
|---|---|---|---|
| Ant. | Post. | Ant. | Post. |
| 18.0 | 17.2 | 17.2 | 15.0 |
| 18.4 | 16.2 | 17.4 | 15.0 |
| 19.0 | 16.2 | 17.4 | 15.2 |
| 18.4 | 17.2 | | |
| 17.6 | 16.8 | | |
| 18.2 | 16.2 | | |
| 109.6 | 99.8 | 52.0 | 45.2 |
| 18.2 | 16.6 | 17.3 | 15.0 |

series, there appears to be no very significant increase in pulse frequency as a result of treatment with pituitary, 1–2,000,000 solution.

Table V. gives a detailed record of the reaction to pituitary, 1 : 200,000 solution, in two individuals. From these results and other similar ones it seems probable that pituitary substance of this strength has a quickening effect upon the contractile vacuoles equivalent to the effect produced by adrenalin. In

general the accelerated pulse rate is of longer duration under the influence of pituitary substance, but dilation of the vacuole is less marked. Vacuoles contracting at an abnormally slow rate, averages 48 and 42, were not stimulated to a normal rate by the

TABLE V.

EFFECT OF PITUITARY, 1 : 200,000.

| Normal Conditions. | | | | 1 Drop Pituitary, 1 : 200,000. | | | |
|---|---|---|---|---|---|---|---|
| A. | | B. | | A. | | | |
| Ant. | Post. | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 17.2 | 16.2 | 13.6 | 16.2 | 17.0 | 14.6 | 8.2 | 15.0 |
| 17.0 | 15.0 | 12.0 | 17.0 | 17.0 | 15.0 | 10.0 | 13.4 |
| 19.0 | 15.0 | 13.0 | 18.6 | 15.0 | 13.0 | 9.6 | 14.4 |
| 16.6 | 15.2 | 13.2 | 18.4 | 14.2 | 12.8 | 11.0 | 14.0 |
| 19.0 | 15.6 | 15.6 | 17.8 | 13.4 | 13.2 | 11.2 | 11.4 |
| 21.0 | 16.2 | 15.0 | 17.8 | 15.4 | 13.0 | 9.4 | 12.8 |
| 20.0 | 16.0 | 12.4 | 18.0 | 16.8 | 13.6 | 9.8 | 11.0 |
| 19.0 | 16.0 | 13.0 | 18.0 | 16.2 | 13.2 | 10.2 | 12.0 |
| 18.0 | 15.8 | 13.0 | 17.4 | 15.2 | 12.4 | 10.4 | 12.0 |
| 19.2 | 16.0 | 14.0 | 17.0 | 15.2 | 14.2 | 10.2 | 12.0 |
| 186.0 | 157.0 | 134.8 | 176.2 | 155.4 | 135.0 | 100.0 | 128.0 |
| 18.6 | 15.7 | 13.4 | 17.6 | 15.5 | 13.5 | 10.0 | 12.8 |

addition of pituitary substance. After being accelerated to 35 and 33 respectively the vacuoles returned to their former abnormal condition. The records taken were not sufficient for the the determination of the lethal or of the optimum solutions.

TABLE VI.

EFFECT OF PINEAL GLAND EXTRACT, 1 : 200,000.

| Normal Conditions. | | | | 1 Drop Pineal Gland 1–200,000. | | | |
|---|---|---|---|---|---|---|---|
| A. | | B. | | A. | | B. | |
| Ant. | Post. | Ant. | Post. | Ant. | Post. | Ant. | Post. |
| 15.2 | 16.8 | 21.2 | 22.6 | 12.2 | 18.0 | 15.6 | 13.8 |
| 15.0 | 18.0 | 20.8 | 20.8 | 12.0 | 15.2 | 15.8 | 13.8 |
| 15.0 | 17.0 | 19.2 | 22.8 | 12.4 | 14.0 | 14.2 | 12.8 |
| 16.4 | 17.0 | 19.6 | 21.2 | 12.6 | 15.0 | 12.4 | 13.0 |
| 14.2 | 18.0 | 18.8 | 22.0 | 13.0 | 15.0 | 13.2 | 13.0 |
| 14.8 | 17.0 | 18.8 | 20.8 | 12.2 | 15.0 | 13.6 | 12.0 |
| 14.4 | 18.4 | 18.8 | 20.0 | 12.6 | 17.0 | 12.8 | 12.6 |
| 15.0 | 19.0 | 19.6 | 20.6 | 13.0 | 16.0 | 14.6 | 12.2 |
| 15.0 | 17.2 | 19.8 | 22.0 | 14.0 | 15.0 | 14.2 | 12.8 |
| 15.0 | 16.8 | 19.0 | 22.0 | 13.0 | 15.0 | 13.8 | 13.0 |
| 150.0 | 175.2 | 195.4 | 214.8 | 127.9 | 155.2 | 140.2 | 129.0 |
| 15.0 | 17.5 | 19.5 | 21.4 | 12.7 | 15.5 | 14.0 | 12.9 |

Table VI. gives a detailed record of the reaction to pineal substance, 1 : 200,000 solution, in two individuals. From these results and other similar ones it seems probable that pineal substance of this strength has a quickening effect upon the contractile vacuoles equivalent to the effect produced by adrenalin and pituitary substance. The pineal substance, however, is much slower in producing acceleration. The results recorded in Table VI. were not observed until an hour after treatment with the solution, when slight dilation of the vacuole also occurred. Vacuoles contracting at an abnormally slow rate, 42 and 38 average showed no significant quickening after treatment with the solution. The records taken were not sufficient for the determination of the lethal or the optimum solutions, but it was noted that the substance in 1–2,000,000 produced no stimulating effects whatever.

Fig. 2 represents graphically the response of Paramecia, with vacuoles contracting at approximately the same rate, to the three different glandular extracts. The average of both vacuoles is shown by each curve. The greatest acceleration is produced by the pituitary substance and the least by the pineal substance.

It is evident from these experiments that solutions of adrenalin, pituitary substance and pineal gland tablets produce an acceleration of pulse frequency in the contractile vacuoles of *Paramecium*. The exact nature of this stimulus is difficult to determine. Each of the solutions employed gave a neutral test, but Rossbach (24) and Degen (6) found that neutral substances produced retardation rather than acceleration. The stimulation cannot be due merely to decreased concentration of the culture medium since the addition of one drop of distilled water to the culture does not produce any marked effect unless the point of evaporation has been almost reached. It seems probable therefore that the stimulation of the vacuoles is produced entirely by the autacoid principles of the glandular extracts. It is questionable, however, whether the stimulation is produced directly by the action of these agents on the vacuole itself, or whether it is the indirect effect of increased metabolism in the entire organism. One is tempted to homologize the effects produced by adrenalin on the vacuoles and on the heart of higher animals, but this seems

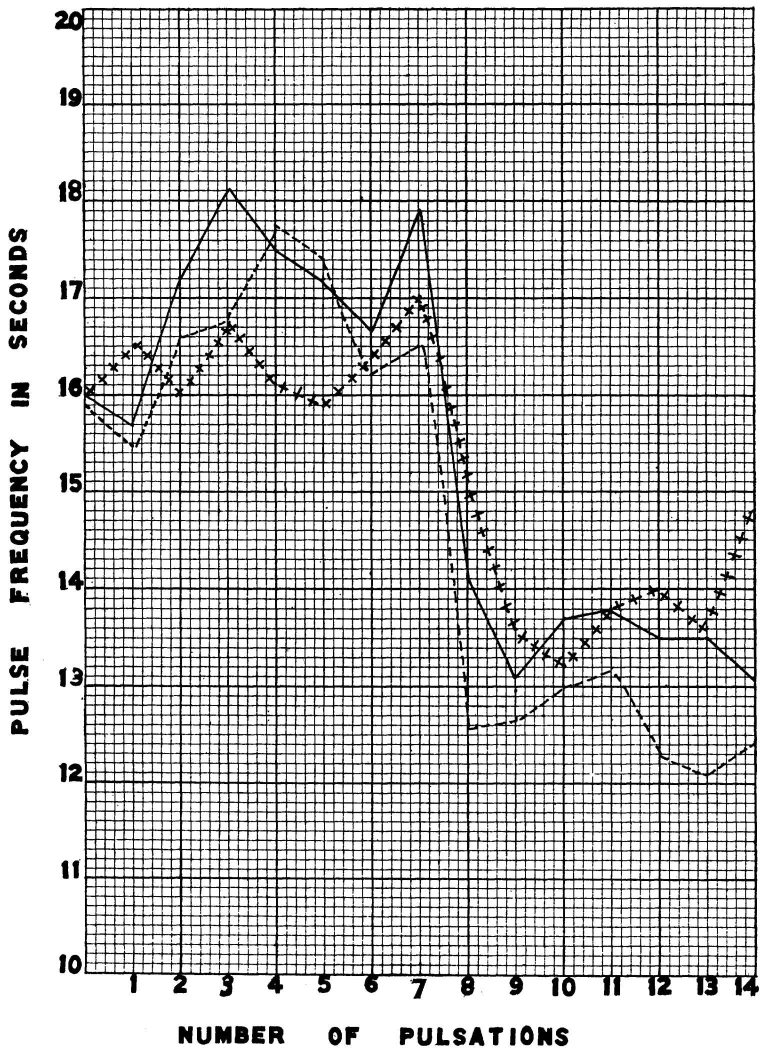


FIG. 2. Increase in pulse frequency following treatment with adrenalin, pituitary substance, and pineal substance, 1 : 200,000 solutions. Ordinates represent pulse frequency in seconds, abscissæ, the number of pulsations recorded. The average of both vacuoles is given. Pulsations 1–7 represent rate under normal conditions, and 7–14 the rate after treatment with the solutions. Unbroken line gives record of individual subjected to adrenalin. Broken line gives record of individual subjected to pituitary substance. Line of crosses gives record of individual subjected to pineal substance.

unwarranted since the presence of a permanent vacuolar membrane is so extremely doubtful. Also it must be noted that pituitary substance appears to produce an even greater quickening of the pulse rate, while in higher animals it has no effect whatever on contractile tissues. Degen (6) it will be remembered claims that the acceleration activated by currents of oxygen is merely a compensation for the increased output of carbon dioxide. A similar explanation seems most plausible for the phenomena produced by the glandular extracts.

Cannon (25) found that inhibition of a strip of intestinal muscle was produced by adrenalin solution as weak as one in twenty mill ons, and Janeway and Park observed inhibition of a strip of coronary artery of the sheep with a solution of one in fifty millions. Takayasu using hemisine, a salt of adrenalin, noted that a solution of two in a million seemed to be the threshold strength for inhibition of the sartorious muscle of the frog. In 1917 Barbour and Spaeth (2) in studying the pharmacological action in single cells found that epinephrin, the active principle of the suprarenal capsule produced contraction of the melanophores of *Fundulus heteroclitus* in all concentrations tested up to one in fifty million. When one considers the far-reaching effects of the various autacoid principles on highly differentiated organisms, it is not improbable that the stimulation of a one-celled organism should be immediately productive of increased metabolism, in compensation for which the contractile vacuoles must become more active in their excretion of waste. The duration of the heightened metabolism will be determined to a certain extent by the concentration of the solution and the neutralizing effect of the digestive fluid. The autacoid principle of adrenalin is believed to be speedily rendered inactive by digestive ferments. To confirm this hypothesis one should find that an increased pulse frequency always occurs with increased division rate. The converse would not necessarily be true, since the metabolic effects might be too temporary to effect the division rate. Schumway (26) noted that under the stimulation of thyroid extract a rapid pulse rate was coincident with a rapid division rate. Much work remains to be done to confirm this hypothesis. Other glandular extracts should be used and individuals in

varying physiological conditions should be experimented upon, while especial care should be taken to correlate the different effects produced on the metabolic activity of the entire organism.

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